Proximity Effects on the Protein Domain Level: Engineering Prolyl Isomerases through Combinatorial Biochemistry

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http://dx.doi.org/10.1016/j.jmb.2013.07.031
Edited by S. Radford

The Role of PPIs

Peptidyl isomerases (PPIs) catalyze the backbone *trans* to *cis* interconversion of AA–Pro (AA: amino acid residue that precedes Pro) bonds. This reaction is particularly important for folding proteins since only proper *cis*-Pro configuration at certain (rather rare) key positions allows for attaining the native and thus functional three-dimensional structure. While some PPIs, such as FKBP12, recognize and catalyze isomerization of short peptides without participating in protein folding, many PPIs are composed of a prolyl isomerase domain that is responsible for the chemistry of the isomerization process and an additional domain that recognizes and transiently binds unfolded or partially folded polypeptide chains. A prime example of this architecture is the trigger factor, which is associated with the ribosome via an additional small attachment site and awaits newly synthesized polypeptide chains to assist in proline isomerization if needed [1,2]. Other PPIs that are also composed of a catalytic site and additional chaperone domain are many proteins from the FKBP family (FKBP12 is rather an exception here) or SurA, SlyD, and parvulins among others [3].

The Additional Chaperone Domain

The effect of this additional chaperone domain is profound. Grafting a chaperone domain to the otherwise highly sequence-specific PPI FKBP12 results in a 200-fold boost in activity in protein folding [4] and to the loss of sequence specificity [5]. It appears that transiently catching polypeptides by interaction with the chaperone domain results in a high local concentration of this substrate that overrides intrinsic specificity. This brings the so-called proximity effect [6] to a higher level. Instead of some precisely arranged amino acid residues that allow for specific binding and positioning and thus high local concentrations of reacting molecules leading to efficient catalysis, we now see this effect on the level of domains and polypeptide chains. The consequence of this shift in dimension is profound. Whereas classical changes of an active site to enhance activity by proximity effects are very difficult to achieve due to the high precision needed, the concept of a chaperone domain feeding the active center of PPI activity appears to be exquisitely robust. With great success did Schmid *et al*. graft chaperone domains onto different PPI domains. A prime example is FKBP12 where not only the chaperone domain of SlyD could be attached [4] but also three other unrelated chaperone domains, namely, from a protein disulfide isomerase from yeast, from SurA, or from a PPI from the periplasm of *Escherichia coli* and even the apical domain of the chaperonin GroEL [7]. In all cases was FKBP12 converted to a bona fide folding assistant.

Properties/Requirements for Chaperone Site

It is worth discussing what makes this chaperone site so versatile. One major contributing factor certainly is the high dynamics of substrate binding and release to avoid an energy well and slowdown of the overall reaction and also rapid screening of substrates. In this respect, PPIs with chaperone domains behave like classical enzymes where rate constants for dissociation are rather high with about 100 s^{-1} [3,8] to allow for fast turnover.

But what makes this chaperone site so universal and how can it adapt to so many different substrate proteins? It was recognized before that chaperone domains in general have a common pattern by which

they establish weak interactions with substrates. For example, the architecture and, more importantly, the substrate binding "clamp" regions of the trigger factor [1,2] and SurA [9] look highly similar. These clamp-like regions can in fact be identified for many chaperone domains; they just appear in different variations concerning the underlying molecular architecture [10]. From sequence comparison and close inspection of different functional chaperone domains (in this issue of the *Journal of Molecular Biology*), Geitner *et al.* extend this view and suggest that "methionine bristles" might play a role for unspecific and only transient interactions with hydrophobic patches of substrate proteins, a very generic concept that could well explain the apparent universality of these chaperone domains. Interestingly, such "methionine bristles" have first been postulated and later discovered in the generic binding sites of the receptors for the signal sequences that destine proteins for secretion [11].

Furthermore, Geitner *et al.* managed to graft the chaperone domain (insert in flap) of SlyD to SurA close to the active site, where in principle other PPIs have their chaperone domain located, but not SurA that contains its chaperone (parvulin) domains remote from the active site. Absolutely astounding, this generates a variant of SurA that even surpasses the wild type in folding activity. Even more surprising does addition of the natural active chaperone domain Par2 further increase the activity and thus clearly shows that the grafted and natural domains do not interfere but rather act synergistically. In all cases, kinetic parameters show again that interaction of substrate with chaperone domains is of moderate strength (K_d about 2–7 μM) and highly dynamic with rate constants for dissociation in the range of 100 s^{-1}.

What Could Be Next?

From the studies of Schmid *et al.*, it becomes increasingly evident that there is a huge potential in the concept of grafting chaperone domains to active sites of enzymes that perform chemistry on polypeptide (partially unfolded) chains. Where could we go from here? There is certainly potential to evolve the chaperone domains for increase in specificity through protein evolution. These evolved cassettes could then be grafted also to other active sites. This is possibly the true potential; would it also work to graft such domains to active sites that modify proteins through phosphorylation, ubiquitination, or other site-specific labeling? The buzzword that comes into mind here is "combinatorial biochemistry". The work by Franz Schmid's laboratory presented in the current *Journal of Molecular Biology* issue and preceding work give good reason for high expectations.

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